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Mr. Lawrence A. Donehower
Laboratory of Tumor Virus Genetics
Bldg 41, Room A114
National Institutes of Health
Bethesda, Maryland 20205

Dear Larry:

I enjoyed our fragmented conversations at CSH but thought it might be useful to provide you with a short outline of what I think might be the best direction to take in your research proposal. I would urge you to focus it around the problem of integration, using perhaps two experimental approaches. The three I favor are described below. At the moment, I think that it's probably too risky to discuss in vitro integration systems in this context, though I believe an in vitro system is a viable longterm goal.

(1) Can viral integration functions be supplied in trans?

Two types of experiments can be described, one in which cells - NIH 3T3 or (better) rat-1 - transfected (or microinjected) with plasmids containing MSV DNA are then superinfected with Moloney strain of MLV, and one in which MLV-infected cells are exposed subsequently (microinjection or transfection) to plasmids containing MSV DNA. In either case, you would score for MSV transformants, then ask whether the presence of MLV increased the frequency of transformation and, more critically, whether integration occurred with precision in the presence of MLV (e.g., at the correct site on viral DNA, with duplication of a 4 base host sequence). The MSV DNA could be unconnected to plasmid DNA, could be in various physical states (linear, closed circle, open circle), and should be tested with one or two copies of the LTR. I have trouble envisioning how to work in the papilloma virus vector: there is the advantage of having persistence of multiple copies of MSV DNA without integration, but I don't know how to score for occasional integration events. I suppose one possibility would be to set the plasmid up so that the integration event would activate or inactivate expression of some marker on the recombinant, but I can't see any realistic way of doing this.

(2) Is the p32 endonuclease required for successful infection?

Here the approach should be in vitro mutagenesis. Using available mapping data and Dennis Schwartz' sequence, it should be fairly easy to make deletions in the p32 region of pol and look for functional virus by transfection. Recovered viruses would, of course, have to be assayed for p32 activities (see papers by Grandgenett). Competent virus without p32 would argue strongly against a role for p32 in infection. It is possible that deletion mutants would not replicate because of problems with processing of polymerase polyproteins, rather than because of a role for p32. We would look at this during "acute" transfection. We should also try making point mutations in the p32 region by isolating an appropriate restriction fragment, subjecting it to mutagenesis with agents such as nitrous acid, than rejoining it to wild type DNA from the rest of the genome for transfection. Virus isolates

(at 35°) would be tested for growth (after cloning) at 35° and 41° to look for ts mutants. A recent assay for polymerase mutants (Goff et al., JV 38:239, 1981) could then be used to screen for (and exclude) ts pol mutants. Other ts isolates would then be assessed for inability to integrate (or make) viral DNA in cells at the nonpermissive temperature and for ts properties of p32.

(3) Is there an identifiable host cell function in integration?

The approach would be to isolate a cell unable to effect integration of Ed's MSV-tk recombinant virus. A tk- line (NIH 3T3, rat-1, or even L cells) would be mutagenized, then subjected to a couple of rounds of infection with Ed's virus to insure infection of all cells. Cells which acquired the tk+ phenotype would be killed with BUdR and survivors retested for susceptibility to conversion to tk+ after another round of infection. Resistant cells would be screened for lack of envelope receptor by infection with VSV(MLV) pseudotypes. Cells susceptible to such pseudotypes would be examined for synthesis and integration of retroviral DNA after infection with MSV, MLV, and possibly others, including MMTV.

When you have thought some of this through, give me a cell or send me a note and we can discuss it further.

Best regards,

Harold E. Varmus, M.D.
Professor of Microbiology and Immunology

P.S. Did you receive the Cell preprint?

HEV:bc